Assortative Mating and the Maintenance of Population Structure in a Natural Hybrid Zone

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ABSTRACT: Understanding the factors that give rise to natural hybrid zones and govern their dynamics and structure is important to predicting the evolutionary consequences of hybridization. Here we use a combination of multigenerational population genetic data, mating patterns from a natural population, behavioral assays, and mark-recapture data within clinal hybrid zones of the genus *Xiphophorus* to test the role of assortative mating in maintaining population structure and the potential for ongoing genetic exchange between heterospecifics. Our data demonstrate that population structure is temporally robust and driven largely by assortative mating stemming from precopulatory isolation between pure species. Furthermore, mark-recapture data revealed that rates of migration within the same stream reach are far below the level needed to support population structure. In contrast to many empirical studies of natural hybrid zones, there appeared to be no hybrid male dysfunction or discrimination against hybrid males by pure parental females, and hybrid females mated and associated with pure species and hybrid males at random. Despite strong isolation between pure parentals, hybrids therefore can act as a conduit for genetic exchange between heterospecifics, which has been shown to increase the tempo of evolutionary change. Additionally, our findings highlight the complexity of natural hybrid zone dynamics, demonstrating that sexual and ecological selection together can give rise to patterns that do not fit classical models of hybrid zone evolution.

Keywords: introgression, mate choice, reproductive isolation, *Xiphophorus*, Poeciliidae.

Introduction

Natural hybrid zones have become increasingly recognized as important sources of evolutionary change (Dowling and Secor 1997; Rieseberg 1997; Barton 2001; Arnold 2006). There are a variety of mechanisms whereby hybridization can affect evolutionary processes. Most common is the scenario in which hybridization produces offspring that are less fit than parental species in some manner (e.g., Burke and Arnold 2001), which is often characterized as aiding the reinforcement of reproductive isolation between species (Butlin 1987). For example, selection against hybrids reduces gene flow between sister species of *Heliconius* that are Müllerian mimics due to the fact that mating between heterospecifics results in offspring with anomalous mimicry patterns and therefore lower fitness of pure individuals that hybridize (Naisbit et al. 2001).

Genetic exchange can also give rise to the formation of new species. After decades of belief that hybridization was often a destructive force, this mode of speciation, and more generally the importance of hybridization in the evolution of plants and animals, has gained increasing recognition (Dowling and Secor 1997; Arnold 2006; Mallet 2007). Two now classic examples involve *Helianthus* sunflowers, in which genetic exchange has enabled hybrids to colonize new environments, and *Heliconius* butterflies, in which strong assortative mating for intermediate wing patterns of hybrids has driven speciation of *Heliconius heurippa*. Furthermore, the advent of next generation sequencing applications to nonmodel systems has revealed that genetic exchange among multiple lineages can play an important role in the evolution of entire genera (Cui et al. 2013). In fact, even when hybridization does not generate new species, gene flow can still facilitate the introgression of adaptive traits from one species to another (e.g., Stein and Uy 2006).

It is also true that extensive genetic exchange can lead to "extinction by hybridization" where pure parental genotypes are replaced by hybrid swarms with mosaic genomes, although this phenomenon is often attributed to effects of anthropogenic disturbance and homogenization of environments (Olden et al. 2004). This phenomenon has been documented or is threatening species in diverse taxa, including fish, mammals, and amphibians among others (Edwards 1979; Abernethy 1994; Childs et al. 1996). Even in cases where entire genetic lineages are not lost, extensive introgression from introduced or otherwise non-

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native species can at least result in the loss of locally adapted genotypes (reviewed in Rhymer and Simberloff 1996 and Olden et al. 2004).

These processes of reinforcement, speciation, and genetic extinction ultimately depend not only on how selection acts on hybrids but also on the extent to which hybrids and the two parental species are each reproductively isolated from one another. The relative strength of reproductive isolation depends on a variety of biotic and abiotic factors. Characterizing the influence of these different factors in hybrid zone dynamics is therefore important to understanding and predicting the evolutionary consequences of hybridization.

Hybrid zones often take on clinal structure, with genotypes transitioning from one pure parental form to the other (see fig. 1 in Barton and Hewitt 1985). The center of clinal hybrid zones is often characterized by strong linkage disequilibrium (LD) indicating an excess of parental genotypes and a deficit of heterozygous hybrid genotypes and giving rise to bimodal structure (Jiggins and Mallet 2000). The maintenance of LD, and more generally the maintenance of hybrid zone structure, has therefore historically been attributed to a balance between inward migration of parentals and selection against hybrids (the so-called "tension zone" model; Barton and Hewitt 1985). However, increasing evidence has mounted in support of other mechanisms that can also generate these patterns of population structure, such as mate choice.

Empirical studies have demonstrated that assortative mating in hybrid zones can lead to the maintenance of LD and bimodal population structure, a pattern traditionally attributed to migration-selection balance (Gregory and Howard 1994; Bridle et al. 2006). Both of these cases come from mosaic hybrid zones in which pure parentals (and hybrids) often coexist within populations across a patchy environment, such that structure deviates to varying degrees from the clinal structure observed in many hybrid zones. A recent theoretical model (M'Gonigle and Fitzjohn 2009) also demonstrated that assortative mating may be a primary driver in the maintenance of bimodal population structure in such mosaic hybrid zones. Importantly, however, this model assumed a homogeneous environment, despite the fact that hybrid zones commonly form along environmental gradients (Fritsche and Kaltz 2000; Nikula et al. 2008; Culumber et al. 2012; Muto et al. 2013) and despite the fact that bimodality in hybrid zones is often associated with environmental selection (Jiggins and Mallet 2000). Their model therefore confirmed that assortativity can be important in mosaic zones but could not address the potential role of assortative mating in zones that conform to clinal structure. This latter point has yet to be addressed by any empirical or theoretical study, to our knowledge. Finally, theoretical studies often assume sexual selection against hybrids (M'Gonigle and Fitzjohn 2009) despite the fact that many hybrids are capable of reproduction (Mallet 2007; Mavárez and Linares 2008), and discrimination against hybrid males or trait combinations is not universal (Stein and Uy 2006; Fisher et al. 2009).

As illustrated by the examples above, characterizing the mechanisms that promote or erode reproductive isolation in hybrid zones is critical to understanding the macroevolutionary consequences of genetic exchange. This is especially true of mating decisions involving hybrids, as the relative preference for or discrimination against hybrids by pure species has implications for reinforcement, hybrid speciation, and the extinction of novel genetic lineages. Behavioral decisions could be particularly important in hybrid zone dynamics given that hybridization often occurs due to a breakdown of premating isolation (Seehausen et al. 1997; Taylor et al. 2006; see also Gröning and Hochkirch 2008 for review). This is especially true given recent theoretical work showing that reproductive isolation can reemerge following temporary disturbance to mating barriers (Gilman and Behm 2011), though empirical evidence of this is still lacking.

Natural hybrids of swordtails (*Xiphophorus birchmanni* and *Xiphophorus malinche*) occur along elevational gradients in replicated stream reaches in eastern Mexico. Hybrids predominate at intermediate elevations, where they outperform pure species that are adapted to opposite ends of an elevational and temperature gradient (Culumber et al. 2012). Despite this apparent selective advantage of hybrids and the potential for sexual selection to favor hybrid phenotypes (Fisher et al. 2009), tests of both linkage disequilibrium and Hardy-Weinberg equilibrium indicate a deficit of heterozygous genotypes (Culumber et al. 2011), with first-generation hybrids almost absent and below expected frequencies. Evidence for hybrid advantage yet a deficit of hybrid genotypes highlights the uncertainty surrounding the mechanisms underlying the dynamics and fate of natural *Xiphophorus* hybrids (Culumber et al. 2011, 2012).

Hybridization is a recent phenomenon (Rosenthal et al. 2003) that is likely due to the disruption of olfaction involving species-specific chemicals signals used in conspecific mate recognition (Fisher et al. 2006). Hybrid populations vary from panmictic hybrid populations (unstructured) to highly structured populations where both parental species and hybrids coexist (Culumber et al. 2011). Panmictic populations where 90%–100% of individuals are introgressed demonstrate that substantial homogenization of pure parental populations has occurred in many localities. However, a deficit of F_1 genotypes dating back to at least 2003 in populations where pure species co-occur (Culumber et al. 2011) suggests that disturbance to reproductive barriers between pure species may be gone in some populations.

Previous behavioral studies have suggested that female mating preferences may play an instrumental role in maintaining population structure. First, both parental species have preferences for urine-borne pheromones of their own species over those of the other parental, which is the primary signal in conspecific mate recognition (Fisher et al. 2006; Cui 2014). Second, females of both species either fail to discriminate between conspecific and hybrid pheromones or prefer the pheromones of hybrids (Verzijden et al. 2012). Third, *X. birchmanni* females prefer visual cues of conspecifics over those of heterospecific *X. malinche* males (Wong and Rosenthal 2006) but prefer visual cues of hybrid phenotypes over conspecifics (Fisher et al. 2009). Finally, though preferences of *X. malinche* females for hybrid male visual cues have not been tested, *X. malinche* females prefer the visual cues of *X. birchmanni* males over those of conspecifics (Cui 2014). The complexity and opposing directions of several of these preferences could therefore have important implications for the dynamics of these hybrid zones.

Here we use a multifaceted approach combining population genetics and genetic analyses of mating patterns, behavioral assays of association preference, and a mark-recapture study in natural populations to estimate the relative contributions of behavioral processes and migration in the maintenance of population structure in *Xiphophorus* hybrid zones. Based on population-genetic patterns, we predicted that premating behavioral barriers reemerged between parental species at some point following the original breakdown in reproductive isolation that led to hybrid zone formation. Drawing on previous work that demonstrated reciprocal adaptation to different thermal regimes, we predicted that inward migration of pure species would be limited and insufficient to maintain subpopulations. Furthermore, we predicted that mating preferences favoring hybrids could maintain gene flow between the two species even in the face of robust behavioral isolating mechanisms between the parental species.

Material and Methods

SNP Genotyping and Subpopulation Dynamics

Genomic DNA was extracted from adult females and embryos or newly born fry from the structured populations Calnali-mid and Aguazarca ($n = 59$ females; appendix, available online; Culumber et al. 2011). All fish were genotyped using three single-nucleotide polymorphism (SNP) markers in three different nuclear introns (LIG1, POLB, and TP53) on different linkage groups as described by Culumber et al. (2011). Adult females were additionally genotyped for the mtDNA control region to differentiate between pure parental females and highly backcrossed hybrids. Using SNP data collected for other studies on the focal populations (Culumber et al. 2011, 2012) together with new data collected here, we calculated the frequencies of *Xiphophorus birchmanni*, *Xiphophorus malinche*, and hybrids in the two populations each for multiple years between 2003 and 2010. These collections were also used to determine the frequencies of male genotypes for tests of mating patterns and to provide quantitative data on the temporal stability of population structure. In order to rule out an influence of misclassification errors, which are inherent to inferring ancestry in hybrid zones, in mating patterns and genotype frequencies, we estimated misclassification rates of genotypes in our data set (table A1, available online).

Test of Mating Patterns

Classification of matings is straightforward based on female and embryo genotypes at the three nuclear SNPs. Each sample was assigned to one of three categories: type-type, F_1, or backcross. This test was more conservative than HWE alone because it directly tests mating patterns. We then used a χ^2 test to evaluate the frequencies of matings among pure species and hybrids. Based on the results of that test, we conducted a second χ^2 test within hybrid females alone. Hybrid female matings were classified as hybrid-hybrid (assortative) or hybrid-parental (backcross, random). For both tests, the frequency of each type of mating expected under random mating was determined based on the actual frequency of male *X. birchmanni*, *X. malinche*, and hybrids in the population as determined by SNP genotyping.

The results did not differ when we used male frequencies from a single year closest to the date of female sampling or the frequencies of males averaged across both populations and all years (data not shown). We therefore used the frequency of each type of male averaged from the eight collections in table A1.

Social Interaction Assays

Adult *X. birchmanni*, *X. malinche*, and their hybrids were collected in early January 2010 at Calnali-mid. Fish were collected, individually marked, and genotyped using the same four SNP markers described above (appendix). Fish were acclimated in mixed-species, mixed-sex aquaria for 2 weeks and then assigned to one of three experimental replicates with equal proportions of male and female *X. birchmanni*, *X. malinche*, and hybrids. Replicates were placed in outdoor mesocosms and allowed to acclimate for 5 days (see appendix). Following the acclimation period, we recorded the position of all individuals on proportional graph paper grids 12 times a day on two con-

secutive days. We digitized grids and used ImageJ software to compute Cartesian coordinates for each individual in each observation. We calculated the pairwise distance between all fish, which prevented pseudoreplication by counting the distance between any two fish in an observation only once. This provided us with pairwise distance matrices for all tanks, trials, and observations.

Since we were interested in the reproductive consequences of hybridization on pure species, we restricted our analysis to female-male pairs. We performed a linear mixed-model ANOVA on pairwise distances between individuals. In the analysis, species pairing type (*birchmanni-birchmanni* [BB], *birchmanni-malinche* [BM], *birchmanni-*hybrid [BH], and so forth for hybrids and *X. malinche* females) was included as a fixed factor with "tank" as a random factor and "observation" was treated as the repeated measure. Having found a significant effect of pairing type, we then performed post hoc comparisons of estimated marginal means of pairwise female-male distances with a Bonferroni correction for multiple tests. Furthermore, in order to rule out the potential for parental species to be drawn to structures on opposite sides of the tanks, which would inflate pairwise distances, we reran the mixed model after excluding all distances greater than 100 cm. This distance cutoff would ensure that distances between individuals at opposite ends of the tank were not included and did not contribute to inflating distances.

Mark-Recapture Estimate of Migration

A mark-recapture experiment was used to estimate movement of individuals among populations in the Río Calnali, the location of our focal structured populations. Fish were captured with minnow traps at Calnali-mid, a 5 × 15-m pool, over a 4-h period in February 2005. All captured individuals greater than 25 mm were marked with subcutaneous elastomer tags. Four months later minnow trapping was conducted in the same pool, as well as in the next pool above (5 m upstream) and the next pool below the site (40 m downstream). We estimated the population size of individuals greater than 25 mm using the recapture rate of marked individuals according to the method of Bailey (1933).

Results

Genotyping and Mating Patterns

Genetic structure of the two focal populations was temporally robust (table A1). Subpopulations of both pure parental species have been maintained in both populations dating back to at least 2003. The mean probability of misclassifying a hybrid as either a pure *Xiphophorus birchmanni* or pure *Xiphophorus malinche* across both populations and all collections was below 5% (table A1). For example, across all 257 individuals collected between 2003 and 2010 from the Calnali-mid population, only two individuals typed as *X. birchmanni* may have been highly backcrossed hybrids. This low rate of error due to misclassification would therefore not influence inferences of mating patterns.

Tests of mating patterns inferred from maternal-offspring genotypes revealed significant deviation from random mating ($\chi^2 = 9.08$, df $= 2$, $P = .011$; fig. 1). This was driven largely by a total absence of F_1 embryos—no interspecific crosses—in the entire sample (0% vs. 11% of expected matings) and associated increase of mating within each pure species subpopulation (53% vs. 38% of expected matings). However, pure species females mated with hybrid males at the frequency expected based on genotype frequencies (47% vs. 52% of expected matings). When restricted to hybrid females alone, mating patterns showed no deviation from random mating ($\chi^2 = 1.20$, df $= 1$, $P = .273$). In other words, hybrid females mated with hybrid and pure parental males at the expected frequency based on the respective male frequencies in the population.

Patterns of Social Interaction in Structured Parental-Hybrid Populations

Assays of social interaction among individuals from a structured population in seminatural mesocosms revealed an effect of species on distances between female-male pairs

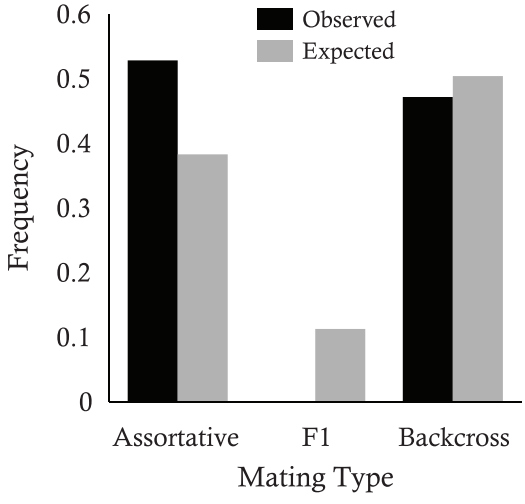

Figure 1: Frequency of assortative (type-type), F_1 (interspecific), and backcross matings inferred from female and embryo genotypes. Observed mating patterns (black) deviated from random expectation of mating frequencies (gray) due to lack of interspecific crosses and increased assortativity of pure parental species ($\chi^2 = 9.08$, $P = .011$).

Table 1: Results from the linear mixed model ANOVA on pairwise distances in open-arena association trials among individuals from a structured hybrid population

Source	Numerator df	Denominator df	*F*	Wald Z	*P* value
Fixed effects:					
Species pair	8	1,189.5	12.864		**<.001**
Random effects:					
Tank				.983	.325

(table 1). Pairwise comparisons of estimated marginal means revealed that pure parental females maintained significantly larger distances to heterospecifics than to consepcific and hybrid males (fig. 2). There was no effect of the random factor "tank." Importantly, the reduced mixed model on only pairwise distances less than 100 cm revealed the same pattern. There was a significant effect of species pairing type ($F_{8,962} = 21.7$, $P < .001$), no effect of tank (Wald $Z = 0.695$, $P = .487$), and pure parental females of both species associated more closely with conspecific rather than heterospecific males (for both $P < .01$). Data underlying figure 2 are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.t7g8f (Culumber et al. 2014).

Mark-Recapture Estimate of Migration

A total of 562 individuals larger than 25 mm were marked at Calnali-mid in February 2005. Four months later, 152/223 (41%) of individuals collected in the same pool were recaptures. Based on the rate of recapture of marked fish, we estimated the population size to be 823 (± 37) individuals larger than 25 mm. Sampling the next pool 5 m upstream produced 1/105 recaptures. Only 1/15 individuals collected in the next pool 40 m below Calnali-mid was a recapture. It is worth noting that Aguazarca, the second structured population in this study, is approximately 800 m downstream from Calnali-mid. Though the mark-recapture study was conducted between February and June, corresponding to a drier time of year in this region of Mexico, extensive sampling has been conducted both upstream and downstream throughout the Río Calnali for six field seasons since the original study, and no more recaptures have ever been observed in the pools immediately up- and downstream at 5- and 40-m distance, respectively, nor in any pool beyond those limits including Aguazarca.

Discussion

Linkage disequilibrium could arise from assortative mating or migration and selection against hybrids. Our data clearly demonstrate that assortative mating contributes to the maintenance of population structure and LD in natural hybrid zones of *Xiphophorus*. In these structured populations, pure species avoid heterospecifics in open-arena association trials, and this is reflected in actual mating patterns. By contrast, mark-recapture data revealed that immigration of pure species is insufficient to maintain pure species subpopulations. Hybrid speciation could result if hybrids are fit and reproductive isolation exists between hybrids and pure species. Females mated at random with hybrid and pure species males, indicating no tendency toward reproductive isolation from pure species. Furthermore, backcrossing to pure species was abundant.

A balance between migration and selection is typically considered the null hypothesis for hybrid-zone dynamics. Strong assortative mating that gives rise to bimodality (preponderance of pure species types) within or among hybrid populations, reinforcing the reproductive boundary between pure species, is often expected to stem from discrimination against hybrids (e.g., Bridle et al. 2006). Our data demonstrate that assortative mating and linkage disequilibrium can be maintained even in the face of frequent mating with hybrids. Therefore, the dynamics of hybrid zones that appear to conform to the tension-zone model may be more complex than commonly believed. This complexity owes largely to the influence of behavioral decisions involving hybrids and to the importance of assortative mating, which likely extends beyond just mosaic hybrid zones.

Previous genotyping of hundreds of individuals showed that F_1 genotypes in the hybrid zones are exceedingly rare and are in fact within the error rate of misclassifying highly backcrossed individuals as F_1s (Culumber et al. 2011, 2012). Not a single F_1 was detected in any of the embryos genotyped in this study. These observations suggest that mating between heterospecifics is extremely rare or nonexistent in contemporary hybrid populations. This was confirmed by examination of mating patterns in structured populations. Of 34 pure species females in our sample, none had mated with the opposite species. Fisher et al. (2006) found that preferences for species-specific olfactory cues were strong in clear water but disappeared in water treated with humic acid, a common substance found in organic waste. Their findings indicated that the breakdown in reproductive isolation between these species likely resulted from disturbance to olfactory communication. This is consistent with work in other northern swordtails that

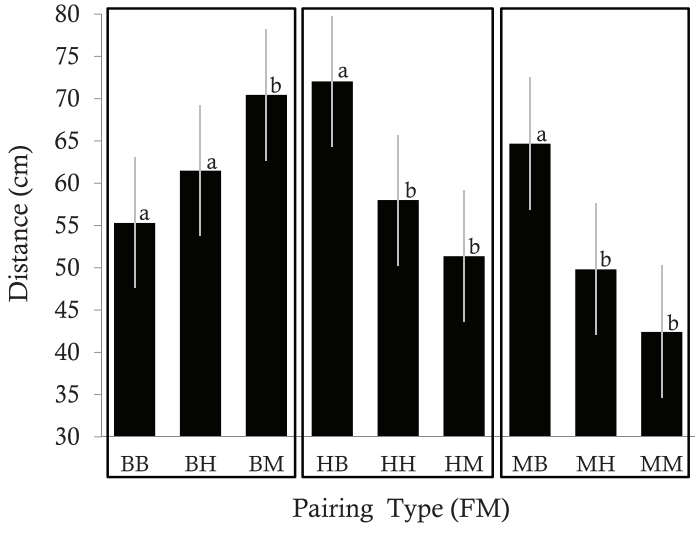

Figure 2: Estimated marginal mean (±SE) pairwise distances from the linear mixed model. The *X*-axis shows species pairing types between *Xiphophorus birchmanni* (B), *Xiphophorus malinche* (M), and hybrid (H) females and males of each species in association trials. The first letter of each pair represents the female's genotype, and the latter represents the male's. Lowercase letters above bars represent distances that were significantly different within each box after correction for multiple tests ($P < .01$). Distances were significantly larger between pure species females and heterospecific males (BM and MB).

has demonstrated strong preferences for species-specific chemical cues (Crapon de Caprona and Ryan 1990; McLennan and Ryan 1997; Fisher et al. 2006). However, our mating pattern results suggest that the disturbance that led to the original breakdown in reproductive isolation may no longer exist, at least in this stretch of river where pure species appear to be isolated at present. Nonetheless, the potential for genetic exchange between pure species persists since both pure species mate with hybrids, which can act as a conduit for gene flow via hybrids even in the face of robust behavioral barriers between hybridizing species.

The mating patterns observed in the natural population were consistent with data from open-arena trials demonstrating that pure-species females from the structured population stayed closest to conspecific males and maintained the largest distances to heterospecific males of the opposite species and intermediate distances to hybrid males. Behavioral association preferences corroborated genetic mating patterns, suggesting that pure species females do not mate with males of the other parental species but regularly mate with hybrid males. This is also consistent with work that suggests that at least some hybrid male phenotypes should be more attractive than those of conspecific males (Fisher et al. 2009). Association trials are a robust and repeatable metric for female preferences and, within poeciliids, predict actual mating outcomes (Cummings and Mollaghan 2006; Walling et al. 2010). Preferences in open-arena trials such as these may be even more indicative of behavior in natural populations as females have access to both olfactory and visual cues, can interact with both sexes and all species types that would be encountered in the wild, and have an open arena with ample space to establish spatial structure.

Sufficiently strong hybrid advantage or assortative mating within the hybrid subpopulation could favor hybrid speciation. For example, assortative mating for wing color patterns has been implicated in hybrid speciation in *Heliconius* butterflies (Mavarez et al. 2006). In our study, by contrast, hybrid females mated at random among the subpopulations, mating equally with pure species and hybrid males. Assortative mating alone is therefore unlikely to promote hybrid speciation in these hybrid zones. Furthermore, at least some hybrid male genotypes are expected to be more attractive to pure species females than conspecific males due to transgressive segregation morphological traits (Fisher et al. 2009). This attractiveness may be reflected in the fact that pure species females associated as closely with hybrid males as they did with conspecifics, though numerical trends favored conspecific males.

Our mark-recapture studied revealed very limited movement of adult individuals within the Río Calnali. Movement to the next pool 5 m upstream was considerably more restricted than movement to the next pool 40 m downstream. This is not surprising given the preponderance of riffles and flumes that separate the larger, deeper pools that swordtails inhabit. Based on the rate of recapture, the population size of Calnali-mid was estimated at

823 individuals. This estimate should be interpreted with caution. The recapture was conducted 4 months after the original marking. This provides opportunity for mortality to occur between mark and recapture. However, this should not result in large error unless mortality is biased between marked and unmarked fish. Additionally, if small individuals grew into the 25-mm size class that was used and were counted as unmarked fish in the recapture, it would cause an overestimation of population size by deflating the true recapture rate. Even assuming some error in the population size estimate, the results of the mark-recapture study suggest very low levels of migration among populations in the Río Calnali. Though we cannot rule out migration of fry, such migration would not explain why genetic structure is observed in embryos of females collected in structured populations.

Selection against hybrids is most often believed to be balanced by inward migration of pure species that continually contribute genes to the lower fitness hybrid gene pool. This concept is particularly important in the context of the maintenance of pure species subpopulations in our focal structured populations. Our mark-recapture experiment provides strong evidence that movement of individuals in the Río Calnali is restricted to relatively short distances. It is important to distinguish between migration and gene flow. Values of F_{ST} among populations within the Río Calnali indicate that some level of gene flow occurs among populations in the Río Calnali, though differentiation was elevated and only marginally nonsignificant (Culumber et al. 2011). Previous data on F_{ST} together with our mark-recapture and mating pattern and behavior data collectively suggest that migration is not sufficient to maintain subpopulations of pure parental species. In the current context of these structured populations, the rate of mating between pure species females and hybrid males should reduce parental subpopulations by more than half within a single generation of mating. The *Xiphophorus birchmanni* subpopulation, for example, would fall below 1% of the total population (~4 individuals) within three generations assuming equal fitness. Yet, the subpopulation has been maintained at an average of 21%, or 173 individuals in Calnali-mid (table 1) based on our population size estimate, over at least seven generations. To maintain such a subpopulation without strong assortative mating would require the immigration of a substantial number of individuals from each pure species every generation. Our mark-recapture data suggest that this is highly improbable.

The dynamics of clinal hybrid zones have traditionally been viewed in terms of the balance between immigration and hybridization of pure species and strong selection against hybrids (natural or sexual) that reinforces barriers to genetic exchange. Our findings highlight the complex nature of hybrid zones, demonstrating that sexual and ecological selection can work in concert to promote the maintenance of stable population structure even without considerable immigration. These results show that substantial mating and gene exchange can occur with hybrids but that female preferences can nonetheless contribute significantly to the maintenance of pure species within populations with important implications for hybrid zone dynamics. Individual behavioral decisions may play a fundamental role in stabilizing hybrid zones.

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